Costs of migration

Schmidt-Wellenburg, Carola Andrea

CHAPTER 3

Energy expenditure during flight in relation to body mass: effects of natural increases in mass and artificial load in Rose Coloured Starlings

Carola A. Schmidt-Wellenburg,
Sophia Engel, and G. Henk Visser

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ABSTRACT

The effect of natural changes in body mass and of artificial manipulation of transported mass on energetic costs of flight was measured and compared in Rose Coloured Starlings *(Sturnus roseus)*. The birds flew repeatedly for several hours in a wind tunnel while undergoing spontaneous variation in body mass and under one of the following treatments: (1) flying unrestrained (henceforth referred to as U), (2) flying with a control harness of 1.2% of their body mass (C), or (3) flying with a harness of 7.4% of their body mass, which was either applied (3.1) immediately before the flight (L_S), or (3.2) at least nine days in advance (L_L). Energy expenditure during flight (e_f, in W), was measured with the Doubly labelled water method. Flight costs in L_S and L_L were not significantly different and therefore were pooled (L). Also the harness itself did not have a significant effect on e_f, *i.e*. there was no difference between U and C flights. Flight costs were allometrically related with body mass (*m*, in g), and the slopes were not significantly different between the different treatments, but flight costs were increased by 5.4% in L compared to C flights ($\log_{10}(e_f)= 0.050 + 0.47 * \log_{10}(m)$ for C flights, and $\log_{10}(e_f)= 0.073 + 0.47 * \log_{10}(m)$ for L flights). For an average bird of 75.6 g, the harness of 7.4% (or 5.6 g) increased flight costs by 0.31 W (3.4%). A spontaneous increase in mass by 5.6 g increased flight costs by 0.35 W (4.2%). The difference in e_f between the three treatments C, L_S and L_L was explained by taking the transported mass, m_{transp} in g, instead of body mass into account. This relation could best be described as $\log_{10}(e_f) = -0.08 * 0.54 * m_{transp}$. Flight costs in Rose Coloured Starlings increased to a lesser extent than expected from interspecific allometric comparison or aerodynamic theory - regardless of whether the increase in body mass occurred naturally or artificially. We did not observe an effect of treatment on relative breast muscle size and wing beat frequency. We propose that the relatively low costs at a high mass are a consequence of immediate adjustments in physiology and/or flight behaviour and not of long-term adaptation to a certain body mass.

INTRODUCTION

Most migratory birds increase their body mass in preparation for long migratory flights, which results in higher flight costs. The extent of the increase of flight costs with body mass is important not only with regard to the actual nutritional status or immediate food requirements, but also to migration distances, strategies and successful completion of migration. Three studies on Red Knots (*Calidris canutus,* Kvist *et al.* 2001), Rose Coloured Starlings (Engel *et al.* 2006) and Barn Swallows (*Hirundo rustica,* Schmidt-Wellenburg *et al.* in press) have shown, that at the individual level carrying natural fuel loads such as fat or protein reserves during long flights is cheaper than expected from aerodynamic theory and from interspecific allometry (Pennycuick 1978, Masman and Klaassen 1987, Rayner 1990, Norberg 1996, Butler and Bishop 2000). It has been proposed that changes in body mass may come along with changes in flight muscle efficiency, both at the interspecific and the intraspecific level (Rayner 1999, Kvist and Lindström 2001, Rayner 2001, Ward *et al.* 2001). These changes in flight muscle efficiency may comprise (1) morphological and/or (2) behavioural changes. Such may include adaptations in the proportion of muscles fibres used, adjusted pectoral muscle size, changes in the muscle composition itself (Bauchinger and Biebach 2001), an improved supply of muscle cells with oxygen and nutrients, or higher density of mitochondria. It is unknown, how much time it takes to develop one of these adaptations. Also strain rate (wing beat frequency and/or wing beat amplitude), effective wing area (by stretching the wings more or less) and consequently wing loading can be modified, or birds could use intermittent flight.

The studies available are all based on spontaneous changes in body mass. Here, we address the question whether birds can instantaneously carry an increased mass with a high efficiency, or whether they have to first develop this capability. We expect that a possible preparation effect (*e.g.* a change in fibre composition or in vascularisation of the muscle) would need time to develop, while modifications of flight behaviour might be immediate. We designed an experiment to quantify the effects of natural changes in body mass and artificial manipulation of transported mass on flight costs. We measured flight costs, body composition, water fluxes, changes in relative breast muscle thickness and wing beat frequency in captive birds flying for several hours in the wind tunnel of the Max Planck Institute in Seewiesen, Germany (see chapter 1, Engel 2005, Engel *et al.* 2006). Individuals flew repeatedly with natural variations in body mass and one of the following four possible treatments: Flying (1) unrestrained (henceforth referred to as "U"), (2) with a light harness, which was applied immediately

before the experimental flight, to control for a possible aerodynamic impairment of the flight by the harness ("C"), or (3) with a load in the harness ("L"), which was either applied (3.1) immediately before the experimental flight (load for a short time, "L_S"), or (3.2) at least 9 days before the experimental flight (load for a long time, "L_L").

We expected flight costs in birds with natural changes in body mass to allometrically scale with mass to the extent measured within species, *i.e.* to a lower extent than predicted from interspecific comparison and aerodynamic theory (Kvist and Lindström 2001, Engel *et al.* 2006, Schmidt-Wellenburg *et al.* in press). We hypothesised that the artificial load increased flight costs relative to unrestrained flights to either (1) a higher extent in case the birds needed internal cues and/or time to "adjust" to the higher mass, or (2) the same extent in case the "adaptations" were immediate.

MATERIALS AND METHODS

Birds, housing, and flight training

Energy expenditure during flight was measured in Rose Coloured Starlings. This is a migratory species with its breeding grounds stretching from the Balkans throughout Central Asia, wintering on the Indian subcontinent. Twice a year, Rose Coloured Starlings cover about 5000 km in mainly daytime migration to overcome these distances (Hudde 1993). We exploited their willingness to perform long flights of several hours during the migration periods, and measured energy expenditure during these flights with the DLW method during March - May 2001, October - December 2001, and March - May 2002.

We measured flight costs in 14 individuals. Four of these birds were purchased in 1999 as nestlings from a breeder and flown in U flights during 2001 (Engel *et al.* 2006), and ten birds were collected as nestlings from a breeding colony on the Crimea peninsula, Ukraine, in 2001, performing U flights in autumn 2001 and C and L flights in 2002. All Rose Coloured Starlings were hand raised at our institute and thus familiar with close presence of humans and handling. The juvenile birds from the Ukraine were regularly trained to fly in the wind tunnel during eight weeks before the first experimental flights. Adult birds had been flying in the wind tunnel before. They were trained for three weeks before the experimental flights in autumn 2001 and for five weeks in spring 2002. During training flights, as well as during the experiments, birds flew in groups of two to three, as the Rose Coloured Starling is a flock-living and also flock-migrating

species. During the weeks before an experimental flight, each bird had performed training flights of at least 90 min duration. All birds used in the experiment were in excellent condition without having any defects in their flight feathers.

The birds were housed in groups of 3 - 5 in aviaries adjacent to the wind tunnel (ca. 1 m x 2 m x 2 m). They received standard food, consisting of insects, beef heart, curd, rusk, and egg *ad libitum*, supplemented with minerals and vitamins, some live mealworms and fresh fruit and salad. They had unlimited access to fresh water and the opportunity to bathe.

The light schedule followed the conditions the birds would experience in the field: during winter, day length (from civil dawn to civil dusk) corresponded to the photoperiod of the wintering quarters in northern India (27.5°N). During spring, it increased weekly until it met the natural day length at 47.5°N in summer, matching the light conditions at the natural breeding grounds as well as at our institute. During autumn, we gradually changed the day length back to the conditions at the wintering grounds. To approximate the spectral composition of natural sunlight, we used Osram Biolux® lamps.

Experimental flights

Fourteen Rose Coloured Starlings flew repeatedly in the wind tunnel of the Max Planck Institute for Ornithology, Seewiesen, Germany. Between the flights, body mass of the birds varied spontaneously between 56.1 and 89.9 g. We artificially increased the mass of the birds in some of the flights by applying a harness. To standardise the additional load relative to body mass, we used a harness equalling 7.4 ± 0.8% of the bird's body mass (with 7.5 ± 0.9% in L_S and 7.2 ± 0.5% in L_L flights). This artificial load of 7.4% was applied, because the pilot phase had revealed, that birds could immediately and sustainably fly with these loads. To control for a possible impairment due to changes in aerodynamics, we used a very light harness without a load ("C"), weighing on average 1.2 ± 0.1% of the bird's body mass (ranging from 0.9 to 1.0 g). The construction of the harness was based on the thigh-method described in Rappole and Tipton (1991; Figure 3.1). The harness rested on the bird's lower back, the synsachrum. Feathers covered the anterior part of the harness and the loops. As the harness was fixed at the bird's thighs (and not with loops around the neck or the wings as, *e.g.* in studies by Nicholls and Warner 1968, Godfrey 1970, Goldspink 1977, Gessaman *et al.* 1991, or Higuchi *et al.* 1996), birds did not run the risk of dying from esophageal blockage (Pekins 1996) and flight ability was not impaired.

Individual birds flew repeatedly (up to nine times) at different body masses (Figure 3.2). During these flights, they were either unrestrained (U), carried a control harness (C), which was applied immediately before the flight, or a loaded harness (L), which was applied either immediately before the flight (L_S) or at least nine days before the experimental flight (L_L). For individuals, the time interval between flights was on average 12.7 ± 6.8 days with a minimum interval of five days.

Birds flew in groups of two or three. They seemed to be flying more at ease in small groups than alone. They did not fly in any specific formation. The starlings could land in the wind tunnel even while the wind was switched on. An observer was standing next to the flight section during the whole flight and, if necessary, prevented the birds from landing or leaving the wind tunnel by waving at it. The mere presence of this person was usually sufficient. The birds were observed during the whole flight. When a bird landed frequently or refused to start again, we let it rest for at least 15 min or took it out of the experiment. Flight speed was constant within all flights. In U flights, it was on average 11.2 ± 1.5 m s^{-1} (SD 0.03 m s^{-1} within a flight). For all other flights, flight speed was adjusted to 11.0 ± 0.04 m s^{-1}. During the flights, birds covered on average 232.4 ± 28.3 km. Air temperature was set to 14.5 ± 0.5°C. The air pressure and relative humidity were not regulated (937.9 ± 8.9 mbar and 58.3 ± 10.3%, respectively).

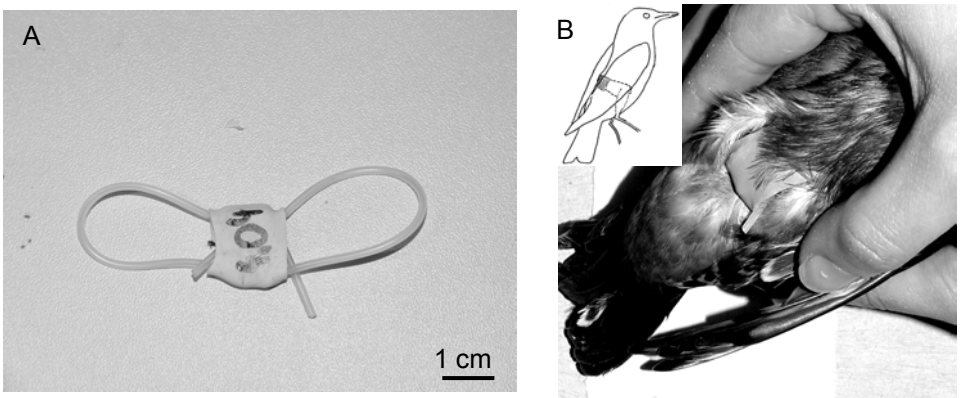

Figure 3.1

(**A**) Harness. As a base, we used a piece of styrofoam for C harnesses and lead for L harnesses. The material was sealed in a shrink hose. This "package" had a size of about 1.5 cm x 1.5 cm x 0.5 cm (w x l x h). As loops to be put around the bird's hips we sewed silicone tubes with a diameter of 0.5 cm onto the shrink hose. (**B**) Harness applied to a bird's back and schematic drawing of the application.

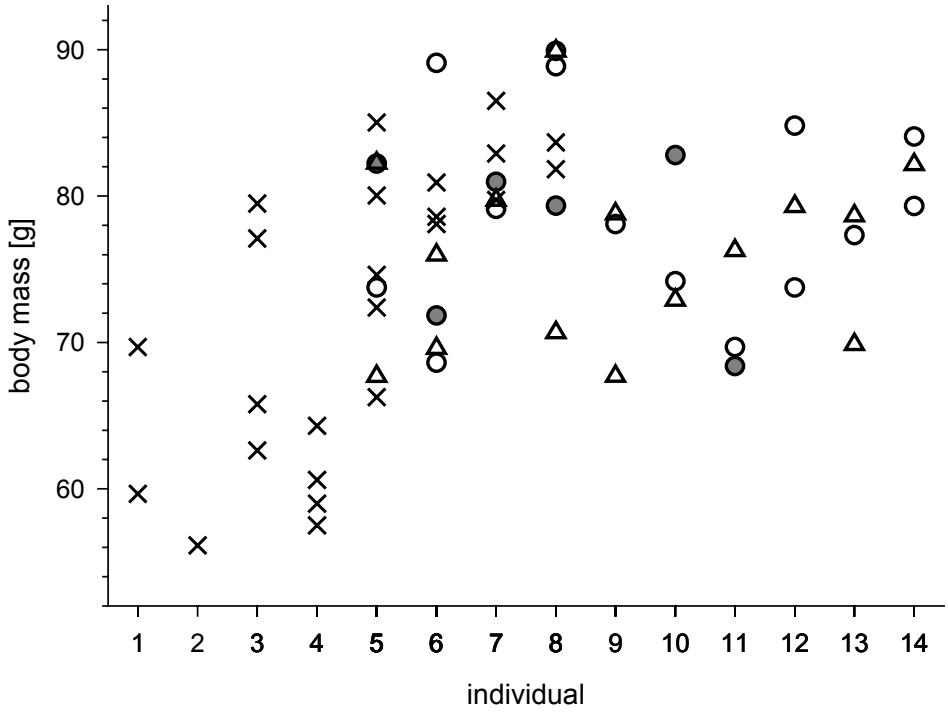

Figure 3.2

Body mass characteristics and treatment of individuals during the experimental flights. We indicated the treatments at natural body mass (in g) for single individuals. Crosses refer to U, triangles to C, open circles to L_S, and grey circles to L_L flights.

Energy expenditure during flight, e_f, and water flux

Energy expenditure during flight, e_f, was measured with the doubly labelled water (DLW) method (Lifson and McClintock 1966, Speakman 1997).

Birds in a postabsorptive state switch faster from carbohydrate to fat combustion, which increases the accuracy of the DLW measurement (Rothe *et al.* 1987). Hence, birds were food deprived since the evening before the experimental flight in most of the measurements. At eight U flights, birds had access to food in the morning before the experiment for about 60 to 90 min (Engel *et al.* 2006). In the morning, shortly after lights on, we took a blood sample of about 60 µl to determine the background concentration in the experimental bird. Afterwards, the bird was injected intraperitoneally with about 0.2 g of a DLW mixture (enriched in ^{18}O by 59.3 atom percent, and in ^{2}H by 34.5 atom percent). The injected dose was quantitatively determined by weighing the

syringe on an analytical balance (Sartorius® BP1215) to the nearest 0.1 mg. After the injection, the bird was placed in a dark box without access to food or water for one hour. We then took another blood sample of 60 µl ("initial sample") from the jugular or the wing vein. From the dilution of the labelled isotopes with the body water pool, we calculated the bird's total body water before the experimental flight ($TBW_{initial}$, in g). We took into account that calculations based on the isotope dilution method tend to overestimate TBW by 1.8% (Speakman *et al.* 2001). In treatments C and L_S, we applied the harness after the blood sample and immediately before the flight. After the flight, 6.0 ± 0.49 h after the initial sample, the "final sample" of 60 µl was taken. Thereafter, we removed the harness in all treatments (C, L_S, and L_L). We injected the bird with 0.11 g DLW afterwards, which was weighed to the nearest 0.1 mg. After one hour rest in the dark box, we took another blood sample ("reinjection sample"), before the bird was released in its aviary, where it had access to food and water again.

The reinjection sample allowed us to calculate TBW at the end of an experimental flight (TBW_{final}), calculate exactly the water influx (rH_2O_{in}, in g d^{-1}) and water efflux (rH_2O_{out}, in g d^{-1}) and thus increase the accuracy of the DLW measurement. We related both TBW and rH_2O to body mass ($TBW_{\%initial}$, $TBW_{\%final}$ (in %), and rH_2O_{in} and rH_2O_{out} in g d^{-1} kg^{-1}, respectively), to account for possible differences in body size. The bird's body mass (*m*, in g) was determined to the nearest 0.01 g on a Sartorius® BL 1500S balance immediately before and after the flight. In the following, we always refer to the average body mass during flight.

All blood samples were subdivided over four capillaries, immediately flame-sealed, and stored at 5°C for the isotope analysis. The isotope analyses were performed in triplicate or quadruplicate at the Centre for Isotope Research according to the method described by Visser *et al.* (2000). Briefly, for each sample $^2H/^1H$ and $^{18}O/^{16}O$ isotope ratios were determined with the CO_2 equilibration method and the uranium reduction methods, respectively (Speakman 1997). The coefficients of variation for ^{18}O and ^2H enrichments relative to the background levels were 1% and 0.75%, respectively. Rates of CO_2 production were calculated as described by Engel *et al.* (2006). As a last step, these values were converted to energy expenditure using a conversion factor of 27.8 kJ l^{-1} (Gessaman and Nagy 1988a). On average the turnover rate of ^{18}O was 3.11 (SD 0.634) times higher than that for ^2H. In consequence, an analytical error of 1% in the measured isotope values would result in an error of about 4% in the calculated energy expenditure.

As the DLW method integrates over the energy spent between the initial and the final blood sample, we corrected for the energy expenditure during non-flight, *i.e.* for resting and handling periods, with the mass-specific metabolic rate of 0.021 W g^{-1} measured in postabsortive Rose Coloured Starlings during daytime at ambient temperatures above 12.9°C by Engel (2005) to yield the flight costs, e_f (in W).

Breast muscle index and wing beat frequency (WBF)

We assessed relative breast muscle size by measuring its elevation relative to the sternum to the nearest 0.01 mm before and after the flight with callipers (Figure 3.3). The first measurement of the breast muscle index was taken before the injection with DLW (henceforth referred to as initial breast muscle index), *i.e.* on average about 7.5 h before the last measurement after the flight. Three measurements were taken at each sampling time and averaged for further analysis. We measured the relative breast muscle thickness in the experimental birds of group C, L_S, and L_L. As a control for non-flight, we also measured it in 8 resting birds, which were kept under the same conditions except for the flight.

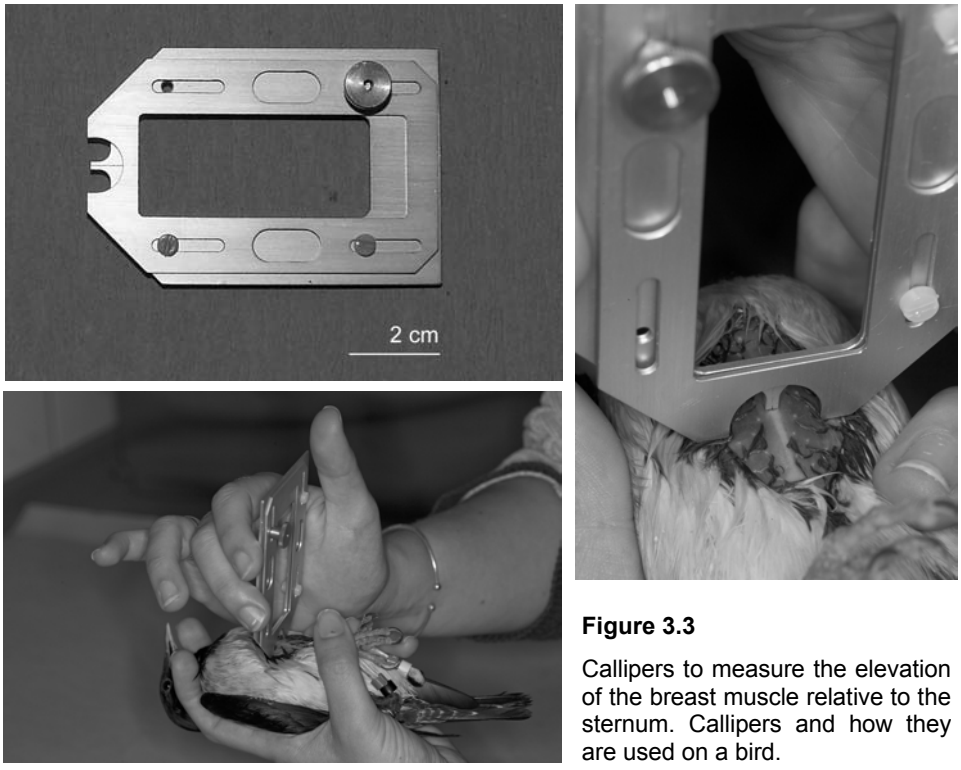

Figure 3.3

Callipers to measure the elevation of the breast muscle relative to the sternum. Callipers and how they are used on a bird.

The resting birds were measured in the morning and after about 7.9 h, a time span comparable to that in the flying birds. During that time, the birds were kept without access to food and water, and, as in the experimental birds, three blood samples were taken. Body mass of these resting birds was on average 90.4 ± 6.9 g during the measurement period.

WBF (in Hz) was visually analysed from video recordings of the flight (watec WAT-902H with 25 frames s^{-1}). For a subsample of flights of treatments C (N= 12), L_S (N= 12), and L_L (N= 6), we analysed flight sequences of 10 sec duration and averaged over five such sequences, as former analyses had revealed that WBF did not detectably change during flight (Engel *et al.* 2006, Schmidt-Wellenburg *et al.* in press). We evaluated the "effective" wing beat frequency, *i.e.* we allowed for periods of bounding and gliding flight.

Statistics

The statistical analyses were performed in SPSS 13.0. The tests were two-tailed, and significance was accepted at $p< 0.05$. Data reported are averages ± SD (between flights). To account for repeated measurements, we tested for effects of body mass *m* (in g) on e_f (in W) in linear mixed models with Restricted Maximum Likelihood (REML). Treatment (U, C, L, L_s, andL_L) was introduced to the analysis as a category, as the mass of the harness was correlated with *m*. The dependent variable was $\log_{10}(e_f)$, individuals were included as random, and $\log_{10}(m)$, treatment, and treatment*$\log_{10}(m)$, and $\log_{10}$(WBF) as fixed factors. The interaction term and $\log_{10}$(WBF) were excluded from the models in a stepwise manner, if they did not significantly contribute to the explained variance.

We tested for effects of the total mass transported in a flight m_{transp} (in g), *i.e. m* plus the mass of the harness, on e_f in linear mixed models with REML as described above. As flight speed was shown to not significantly effect e_f in the U flights (Engel *et al.* 2006), it was not included into the models. The effects of treatment and body mass on $\log_{10}$(WBF) were tested in a separate linear mixed model, analogous to the model described above.

RESULTS

We measured energetic costs of flight, e_f, in 65 flights by 15 individuals. Flight behaviour, *i.e.* if birds flew non-stop or interrupted the flight, had been shown to have a strong impact on the estimate of e_f (Schmidt-Wellenburg *et al.* in press). We therefore excluded interrupted flights (*i.e.* four U, one C, and one L_S flight) and restricted the analysis to the 59 non-stop flights. In these flights, birds flew on average for 5.8 ± 0.5 h, or during 94.9 ± 2.0% of the time measured with the DLW method. The estimated energy expenditure during non-flight accounted on average for 1.0 ± 0.4% (range 0.3 - 2.3%) of the total amount of energy spent during a session.

24 Flights by eight individuals were performed without an artificial load (U). Mean body mass during these flights ranged from 56.1 to 86.5 g and was on average 72.6 ± 9.9 g. e_f during U flights was on average 8.08 ± 0.75 W, ranging from 6.58 to 9.17 W. Ten individuals flew in C treatments (15 flights). Mean body mass during these flights was on average 76.1 ± 6.4 g, ranging from 67.7 to 89.9 g, and birds spent on average 8.56 ± 0.60 W during flight (ranging from 7.60 to 9.42 W). An artificial load was carried by ten individuals in 20 flights, with ten birds flying 14 times in treatment L_S and six individuals flying six times in treatment L_L. Body mass during L flights was on average 78.8 ± 6.7 g (ranging from 68.4 to 89.9 g), and e_f was 9.17 ± 0.82 W (ranging from 7.72 to 10.75 W).

In general, there was a positive association of e_f with body mass (Figure 3.4). In the presence of $\log_{10}(m)$, the interaction term $\log_{10}(m)$*treatment did not significantly reduce the observed variance of $\log_{10}(e_f)$ and was therefore excluded from the models. C flights did not differ significantly from U flights, *i.e.* the harness itself did not affect e_f (Table 3.1.a). Flight costs in the U and C flights scaled as (Table 3.1.b)

$$\log_{10}(e_f) = -0.149 + 0.57 * \log_{10}(m). \qquad \text{(equation 3.1)}$$

For further analyses of the energetic costs of flight, we excluded U flights, as they were not performed in all birds. We therefore took the C flights as the baseline for comparisons between the different treatments of the ten other birds (Figure 3.2).

We separately tested for an effect of the duration of the load application by comparing L_S and L_L flights. We did not detect a difference in the allometric scaling between the two treatments (Table 3.1.c). The two groups were pooled to form the L group for further analysis. In a next step, costs of flight were

compared between L and C treatments. The slopes were not significantly different, but birds in treatment L used more energy during flight (Figure 3.5 A, Table 3.1.d). From this simultaneous analysis, the relationship between energy expenditure during flight, e_f (in W), and body mass, m (in g), is best described by

$$\log_{10}(e_f) = 0.050 + 0.47 * \log_{10}(m) \qquad \text{(equation 3.2.1)}$$

for C flights, and by

$$\log_{10}(e_f) = 0.073 + 0.47 * \log_{10}(m) \qquad \text{(equation 3.2.2)}$$

for L flights. This indicates an elevation of e_f over the entire mass range by 5.4% in L flights (Table 3.1.d, Figure 3.5 A).

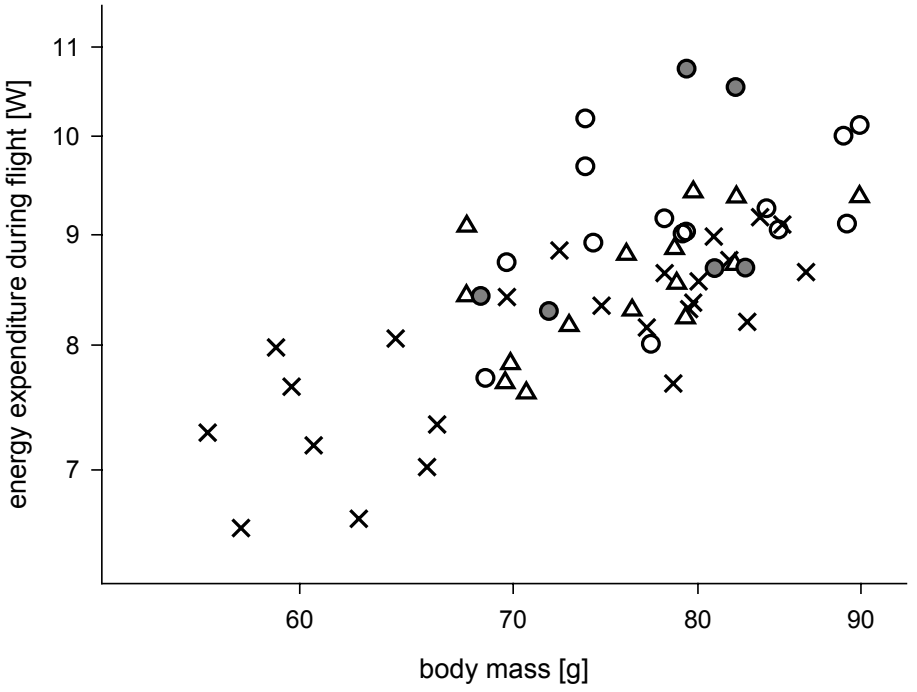

Figure 3.4.

Energy expenditure during flight (e_f, in W) in relation to body mass (m, in g) on a double logarithmic scale. Crosses refer to U, triangles to C flights, open circles to L_S, and grey circles to L_L flights.

Table 3.1

The effect of $\log_{10}(m)$ and treatment on $\log_{10}(e_f)$. N_U= 24 in eight individuals, N_C= 15 in ten individuals, N_{LS}= 14 in ten individuals, N_{LL}= 6 in six individuals;, N_L= 20 in ten individuals.

	Explanatory variable	Data	Estimated effect	F	95% CI	p
a)		U, C				
	intercept		-0.108	F1, 28.2= 0.434		
	$\log_{10}(m)$		0.546	F1, 27.9= 44.136	0.377 - 0.714	<0.001
	treatment			F1, 35.9= 2.399		0.130
	U		0[a]			
	C		0.013			
b)		U, C				
	intercept		-0.149	F1, 27.3= 0.933		
	$\log_{10}(m)$		0.571	F1, 27.1= 47.390	0.401- 0.741	<0.001
c)		L_S, L_L				
	intercept		0.442	F1, 10.8= 1.905		0.195
	$\log_{10}(m)$		0.274	F1, 10.8= 2.629		0.134
	treatment			F1, 9.0= 0.066		0.802
	L_L		0[a]			
	L_s		-0.003		-0.029 - 0.023	
d)		C, L				
	intercept		0.050	F1, 31.1= 0.054		
	$\log_{10}(m)$		0.468	F1, 31.1= 10.955	0.180 - 0.757	<0.01
	treatment			F1, 25.7= 5.637		<0.05
	C		0[a]			
	L		0.023		0.0003 - 0.043	

[a] used as reference for the other treatment effects

When we accounted for the mass added by the harness (1.2% or 7.4% of the birds' natural body mass) and related e_f to the actually transported mass m_{transp} (in g) instead of the natural body mass m, we did not observe any significant effects of treatment on e_f (Table 3.2, Figure 3.5 B). The relation was best described as

$$\log_{10}(e_f) = -0.080 + 0.54 * \log_{10}(m_{transp}). \quad \text{(equation 3.3)}$$

Table 3.2.

The effect of $\log_{10}(m_{transp})$ and treatment on $\log_{10}(e_f)$.

Explanatory variable	Estimated effect	F	95% CI	p
intercept	0.053	$F_{1, 31.3}$= 0.048		
$\log_{10}(m_{transp})$	0.466	$F_{1, 31.3}$= 10.929	0.178 – 0.753	<0.01
treatment		$F_{1, 26.8}$= 0.984		0.330
C	0[a]			
L	0.011		-0.012 – 0.033	
intercept	-0.080	$F_{1, 32.0}$= 0.118		
$\log_{10}(m_{transp})$	0.538	$F_{1, 32.0}$= 19.712	0.291 – 0.785	<0.001

[a] used as reference for the other treatment effects

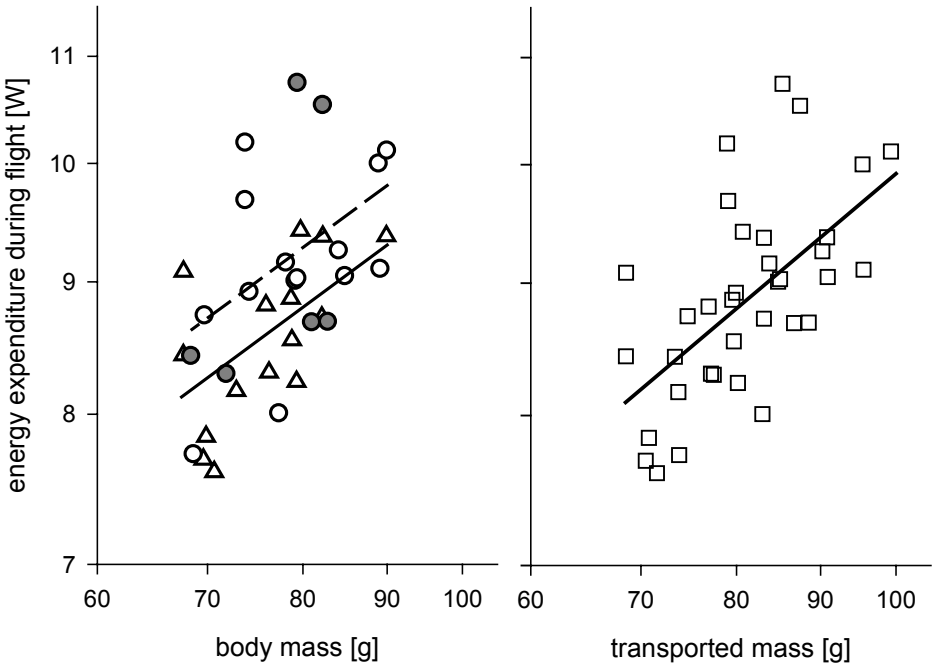

Figure 3.5

Energy expenditure during flight (e_f, in W) in relation to (**A**) body mass *m* (in g) and to (**B**) transported mass m_{transp} (in g) on a double logarithmic scale. (**A**) Triangles refer to C, open circles to L_S, and grey circles to L_L flights. The regression fitted to C flights is solid (equation 3.2.1) and the one fitted to L flights is dashed (equation 3.2.2). (**B**) The regression is fitted to all data, represented as squares (C, L_s and L_L; equation 3.3).

TBW was on average 45.1 ± 3.5 g before and 43.2 ± 3.4 g after the flights (N= 59). Consequently, water efflux, rH_2O_{out}, was higher (31.3 ± 7.0 g d^{-1}) than water influx, rH_2O_{in} (23.0 ± 4.5 g d^{-1}). Neither TBW when expressed as a percentage of the birds' body mass ($TBW_{\%}$) nor mass-specific water flux (in g d^{-1} kg^{-1}) differed between the treatments (Figure 3.6).

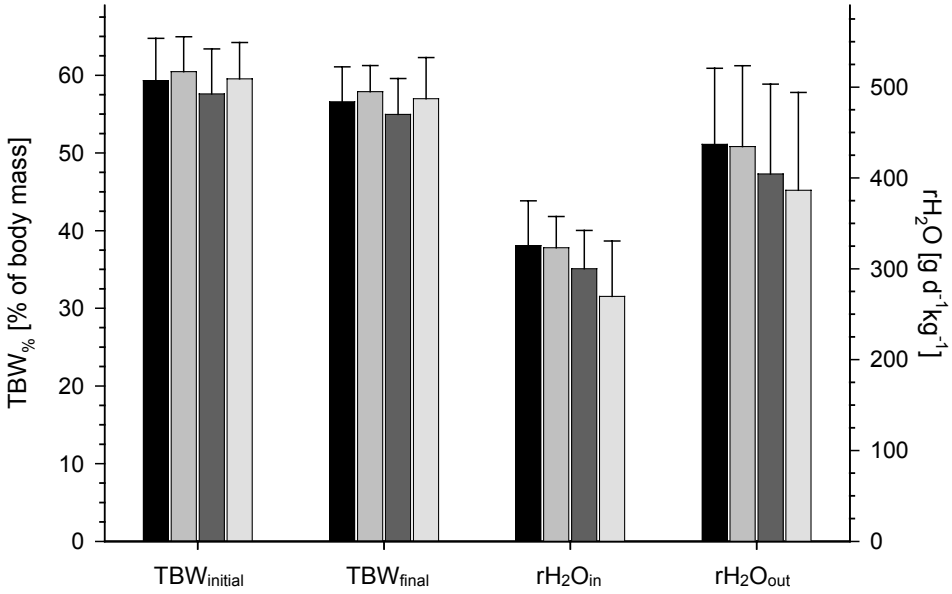

Figure 3.6

Total body water ($TBW_{\%initial}$ and $TBW_{\%final}$, in % of body mass) and mass-specific water flux (rH_2O_{in} and rH_2O_{out}, in g d^{-1} kg^{-1}). Depicted are the averages (+ SD) per treatment, with black bars for U, grey for C, dark grey for L_S and light grey for L_L flights.

The initial breast muscle index was on average 5.34 ± 0.57 mm in flying (N= 35) and 5.57 ± 0.42 mm in resting birds (N= 8). With regard to a possible adjustment to an increased load, we did not observe a difference in initial breast muscle index between L_S and L_L treatments (Mann-Whitney U= 40.0, Wilcoxon W= 61.0, *Z*= -0.165, p> 0.05). After either a flying or a fasting session, the breast muscle index had decreased significantly (Figure 3.7, Wilcoxon signed ranks test, *Z*= -4.842, p< 0.001, N= 43). In flying birds, the level of decrease was not significantly different between L and C. There was also no difference in the decrease between flying birds and resting birds. All birds exhibited similar changes in the breast muscle index irrespective of the treatment.

The loss of body mass relative to the initial body mass during a flying or a fasting session amounted in U, C, L_S, L_L and resting birds on average for 6.81 ± 1.21%, 7.32 ± 0.83%, 7.60 ± 1.03%, 7.49 ± 1.18%, and 3.73 ± 0.70%, respectively. There was no statistically significant difference with regard to the treatments of flying birds on body mass loss (treatment (C and L): $F_{1, 56.0}$= 3.65, p= 0.061). However, mass loss was significantly lower in resting than in flying birds ($F_{1, 35.3}$= 102.65, p< 0.001, 95% CI 2.96 - 4.45%).

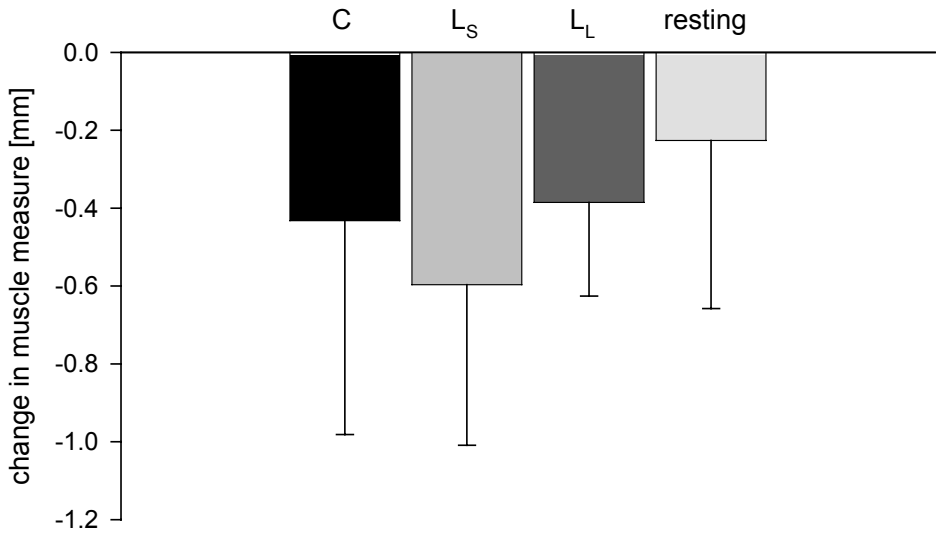

Figure 3.7

Changes in breast muscle index (in mm). Average changes (-SD) during C (black), L_S (grey), and L_L flights (dark grey) and in resting birds (light grey).

Effective wing beat frequency WBF was on average 8.32 ± 0.82 Hz in C flights (N= 12), 8.88 ± 1.06 Hz in L_S (N= 12) and 8.33 ± 0.64 Hz in L_L flights (N= 6; Figure 3.8). WBF did not explain e_f (Pearson Correlation= 0.320, p> 0.05, N= 30). It was not dependent on treatment, but on mass alone. Body mass *m* and transported mass m_{transp} yielded similar results, and the percentage of the explained variance was not different between the models. The allometric scaling of WBF can be best described as

$$\log_{10}(\text{WBF}) = 0.20 + 0.38 * \log_{10}(m_{transp}). \qquad \text{(equation 3.4)}$$

The 95% CI for the factor was 0.036 – 0.723 ($F_{1, 24.7}$= 5.18, p< 0.05).

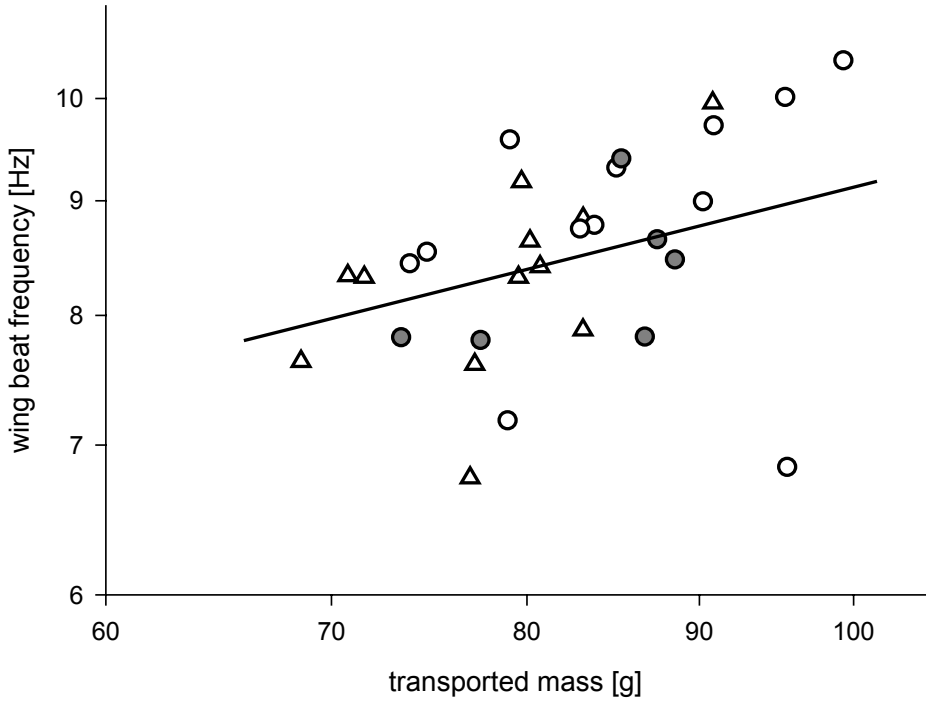

Figure 3.8

Wing beat frequency (in Hz) in relation to transported mass (in g) on a double-logarithmic scale. Triangles refer to C, open circles to L_S, and grey circles to L_L flights. The drawn line refers to equation 3.4.

DISCUSSION

Energy expenditure during flight, e_f, allometrically scaled with body mass. We did not observe a significant difference in flight costs in unrestrained (U) and control harness (C) flights after taking body mass into account. This indicates that there was no effect of the harness itself on e_f. There were no detectable differences between the level of flight costs of L_S and L_L. Apparently the duration of the harness exposure did not affect flight costs. Energy expenditure during flight, e_f, allometrically scaled with body mass to the power of 0.47. This exponent did not significantly differ between flights with a control or a loaded harness. Flight costs were 5.4% higher in L_S and L_L compared to C flights. If the additional load in L flights were a natural increase in body mass, with equation 3.1 we would predict an elevation of 4.5% instead of the 3.4% predicted from

equation 3.2.2. This difference is very small indeed. Thus, flying with a metabolically inactive load appeared to be about as expensive as flying with a naturally increased body mass. Flight costs apparently increased with transported mass m_{transp} regardless of how the mass was increased.

Flight costs

The observed exponents of the scaling of e_f with mass of 0.57 and 0.54 (equation 3.1 for m and equation 3.3 for m_{transp}) lie in the range of other intra-specific measurements in birds flying for prolonged periods in a wind tunnel: Kvist *et al.* (2001) recorded an exponent of 0.35 in Red Knots. Flight costs increased with $m^{0.55}$ in Rose Coloured Starlings (Engel *et al.* 2006), and with $m^{0.58}$ in Barn Swallows (Schmidt-Wellenburg *et al.* in press). These results contrast both with aerodynamic theories and with interspecific allometric comparisons. Aerodynamic theories predict a scaling exponent of 1.1 - 1.6 (Pennycuick 1975, 1978, Norberg 1990, Rayner 1990, Norberg 1996), interspecific allometry observed scaling exponents ranging from 0.7 to 1.9 (Masman and Klaassen 1987, Rayner 1990, Norberg 1996, Butler and Bishop 2000, Videler 2005). At the species level, e_f increases to a lower extent than expected from theoretical considerations or extrapolated from interspecific allometry.

With regard to the harness applied, we had expected flight costs to increase, because the harness affects aerodynamics during flight, especially with regard to the surface of the back and positions of the back feathers. However, we neither observed a difference between flight costs of U and C flights, nor did we detect any difference between the treatments, when e_f was related to m_{transp}. The harness was flat, had an even surface, and it was soon mostly covered with feathers. The thigh-loops did not impair the wings during flight, and furthermore, the load was placed at the barycentre, comparable to natural fuel loads.

A load of 7.4% of the birds' body mass is a rather small mass change compared to spontaneous changes of about 40% in the Rose Coloured Starling (chapter 1). During the pilot phase we applied loads of up to 20%, but some birds did not fly immediately or refused to fly for longer periods. As we wanted to compare L_S and L_L, we did not train the birds to fly with a higher load and restricted the harness to 7.4%.

Other studies on short flights have shown that birds accepted high loads of 20 - 27% (Nudds and Bryant 2002, Hambly *et al.* 2004a). In very short burst flights of 0.4 s, hummingbirds lifted maximal loads close to twice their body mass

(Chai and Millard 1997). These short flights are comparable to feeding flights rather than to migratory flights. There are no data on such high loads carried during long flights.

The effect of an artificial load on flight costs has been measured in long flights in two studies on pigeons (*Columba spp.*, Gessaman and Nagy 1988b, Gessaman *et al.* 1991) and in short flights in Zebra Finches (*Taeniopygia guttata*, Nudds and Bryant 2002) and Cockatiels (*Nymphicus hollandicus*, Hambly *et al.* 2004a). In pigeons, energy expenditure during flight was not increased when carrying a harness of 5% of the body mass (Gessaman *et al.* 1991). Flight speed in these birds was increased compared to control flights (they spent 21 - 26% less time flying). In another study on pigeons carrying a harness of 2.5 or 5% of their body mass (Gessaman and Nagy 1988b), energy expenditure was increased and flight speed reduced by 15 - 27% compared to control flights without a harness. Reducing or increasing flight speed was not an option for our birds flying in the wind tunnel with a set velocity.

In measurements of short flights of Zebra Finches flying with a harness of 27% of their body mass, e_f was the same as in control flights (Nudds and Bryant 2002). Take-off speed in these birds was reduced, and mass loss during 28 h elevated. Hambly *et al.* (2004) observed no changes in e_f in Cockatiels flying with a load of 5 - 20% during 2 min. Flight speed was reduced in flights with a 15% harness, and WBF was increased in flights with a 20% harness. It is not yet understood why measurements of e_f in pigeons, Zebra Finches and Cockatiels did not reveal an increase with load. It might be a phenomenon of short flights. In the three studies, birds could choose their own flight speed, whereas we let them fly at a given speed. Flight costs in Rose Coloured Starlings are not dependent on flight speed (Engel *et al.* 2006), and it is thus unlikely that speed played a role.

Breast muscle index

The idea of a rapid "adjustment" to a load is also supported by the breast muscle index. Birds in L_S and L_L did not significantly differ in the breast muscle index before a flight. So birds, which carried a harness for a prolonged period of time, did not detectably adjust breast muscle size.

During a flying or fasting session, breast muscle size decreased in all treatments (C, L_S, L_L, and resting). We did not observe an effect of treatment (C, L_S or L_L). The decrease in breast muscle measure during a session appears to be related mainly to food and water deprivation, as it also decreased in resting birds. We

did not further investigate if the pectoral muscle decreased due to the combustion of specific resources such as protein (Bauchinger and Biebach 1998). The breast muscle is of course not the only source of energy: body mass in flying birds decreased twice as much as in resting birds.

Water turnover

Water turnover in our study was about the same in all treatments. Gessaman *et al.* (1991) recorded higher water loss in birds flying with a 5% harness or with a control harness than flying unrestrained. Their measurements were based on DLW as well. However, their estimates of rH_2O_{out} were difficult to interpret, as turnover rates were low, and birds were displaying a wider range of behaviours during the measurement, such as sunbathing, whereas our birds flew for 95% of the time measured.

Wing beat frequency

We analysed WBF as a possible behavioural adjustment to an increased body mass. Although it was positively correlated with body mass, it neither significantly explained e_f nor was it related to treatment. An increase in transported mass alone, be it natural or artificial, resulted in higher WBF. The observed exponent of the correlation of WBF and m_{transp} of 0.32 is similar to that observed within species (0.38 in Barn Swallows; Schmidt-Wellenburg *et al.* in press) and proposed for individuals (0.5; Pennycuick 1996). We propose that possible changes in flight behaviour are not based on changes in WBF but may rather be related to changes in stroke amplitude or effective wing area.

Implications

This is the fourth study (Kvist and Lindström 2001, Engel *et al.* 2006, Schmidt-Wellenburg *et al.* in press) showing that energetic costs during flights of several hours increase with body mass to a lower extent than expected from interspecific comparisons and aerodynamic theories. The low increase in flight costs is reached immediately after an additional load of 7% of the body mass had been added. Apparently no long-term physiological changes or internal cues are necessary to optimise flight costs at a higher mass in Rose Coloured Starlings. It may rather be short-term changes such as in flight behaviour. Adaptations such as in flight efficiency still may operate on a larger time-scale.

Wing beat frequency scaled with the transported mass as expected, and we hence assume that stroke amplitude or effective wing area may be altered.

Rayner (2001) hit the mark when he wondered "why a bird carrying a small fuel load can't operate at the high efficiency that it can reach when carrying a large load". We speculate that birds can indeed always fly with a high efficiency and that efficiency does not change with mass, at least during the migratory season. It might just be that aerodynamic considerations do not meet the reality of a flying bird and overestimate the effect of an increased mass on flight costs. An additional mass, which is either evenly distributed over the body or with an emphasis on the barycentre, may even enhance lift of the body and tail and only slightly impair drag (see Dolnik 1995). Our study thus strengthens the need for a novel aerodynamic theory of avian flight (Videler 2005).

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In case the falcon flaps its wings and intends to take off, the falconer should take it onto his hand, and let it chew on bones, spray the bird with cold water, or let it bathe.
(fol. 131 v)